Applicants have added dependent claims 26, 30, 34, 38, 42, 46 and 50 to require that the "antigenic portion" of the transcription associated biomolecule is "not endogenous to said host cell." The nucleic acid fragment encoding the "antigenic portion" is constructed as a peptide bait, to screen for single chain antibody fusion reagent within the host cell (see specification at page 22, line 27 to page 28, lines 1-3). The claimed invention also contemplates a screening protocol that uses novel vectors to express antigen binding domains of immunoglobulins, which target endogenous transcription associated biomolecules within the host cells (page 15, lines 26 to page 16, lines 1-6). Such endogenous biomolecules do not contain or are not fused to the DNA binding domain peptide, and they therefore will not interfere with the successful screen. This is so because abundant expression of the fusion reagent by host cells ensures sufficient amounts of the reagent to bind chimeric DNA binding domain/transcription associated biomolecules.

In subparagraph (e) of claim 1, Applicants have qualified the recited "binding" to indicate that the prescribed "chimeric...peptide...may bind to said antigenic portion" (emphasis added).

At the interview, Applicants directed the Examiner Visintin *et al.*, *Proc. Nat'l Acad. Sci. USA* 96: 11723 (1999) (enclosed copy), who used a single chain antibody fusion reagent of the present invention. More specifically, Visintin *et al.* showed that not all sFv fragments isolated from the conventional phage display library are able to bind their antigen when expressed intracellularly, in a two hybrid system. According to the authors, "a selection step after the *in vitro* stage is necessary to separate the '*in vitro* only' binders, from those that also can bind *in vivo*." *Id.* at 11727. In other words, Visintin *et al.* demonstrated that Applicants' claimed invention offers an advantage over conventional phage-display methodology, the results of which do not correlate predictably with intracellular antibody-antigen binding interactions.

Claims 1-23 are pending in the application. Claims 8 and 23 are cancelled, without prejudice or disclaimer. These claims are duplicative of claims 1 and 22, respectively. Claims 1-7 and 8-22 are amended and claims 24-53 are added, to set forth the nature of the claimed invention more clearly. Accordingly, claims 1-7, 8-22, and claims 24-53 are presented for reexamination and reconsideration.

The proffered claim revisions are supported throughout the original specification and generally conform to suggestions advanced at the April 25th interview. Because the present amendment does not introduce new matter, Applicants request its entry and a favorable disposition of the claims by Examiner Unger.

The Examiner is hereby authorized to charge any deficiency or credit any overpayment to our Deposit Account No. 19-0741.

Respectfully submitted,

Date 25 June 2002 By S. A. Bent

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MARKED UP VERSION SHOWING CHANGES MADE

1. (Amended) A method of screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcription associated biomolecule [*in vivo*] within a host cell, comprising:

(a) cloning a first nucleic acid fragment [which encodes] that codes for a [peptide DBD] DNA-binding domain peptide of a transcription [factor] activator into [an] a first expression vector to yield a construct (1), [such that the DBD may be expressed in a bio-active form and bind a corresponding] wherein said DNA-binding domain peptide binds to a DNA regulatory sequence binding site [in the heterologous host cell,];

[fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule into construct 1, in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2),]

(b) fusing a second nucleic acid fragment into said construct (1), in the same translation reading frame as said first nucleic acid fragment, to yield said first expression vector containing a construct (2) that encodes a chimeric DNA-binding domain/transcription associated biomolecule, wherein said second nucleic acid fragment codes for an antigenic portion of said transcription associated biomolecule that is sufficient to generate antibody capable of binding to said transcription associated biomolecule;

(c) providing said host cell containing a detectable gene that is under the transcriptional control of the DNA regulatory sequence binding site for said DNA-binding domain peptide;

(d) cloning a third nucleic acid fragment that codes for a single chain antibody [an sFv library] into a second expression vector [a DNA construct] to yield a construct (3), [such that a] wherein said single chain [monoclonal antibody may be] antibody is expressed in a bio-active form [and bind a corresponding antigen in a heterologous host cell,] that may bind to said antigenic portion;

(e) fusing a fourth nucleic acid fragment [which encodes] that codes for a trans-activation peptide into said construct (3), in the same translation reading frame [of] as

the third nucleic acid fragment [which encodes the single chain monoclonal antibody,] to yield [a] said second expression vector containing a construct (4), [such that a resulting] encoding a chimeric [sFv] single chain antibody/trans-activation peptide [may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell,] that may bind to said antigenic portion;

[providing a heterologous host cell harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2,]

(f) introducing [constructs 2 and 4] said first and second expression vectors into [the heterologous] said host cell [harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2], such that both [constructs may be] vectors are expressed[,]; and

[identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule in vivo by selecting for expression of the detectable gene]

(g) monitoring the expression of said detectable gene, whereby detecting said expression indicates that said fusion reagent does bind said antigenic portion within said host cell upon detection of said expression.

2. (Amended) The method [of] according to claim 1, further comprising fusing at least one nucleic acid fragment, [which encodes] that codes for an intracellular targeting signal peptide, into said construct (4), in the same translation reading frame [to the] as said third nucleic acid fragment [which encodes the single chain monoclonal antibody in construct 4, to yield a modified construct (5), such that a resulting single chain monoclonal antibody fusion reagent may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell], to yield a construct (5), wherein said intracellular targeting signal peptide directs the expression of said single chain antibody to a cellular compartment.

3. (Amended) The method [of] according to claim [1]2, [further comprising fusing at least one nucleic acid fragment, which encodes an intracellular targeting signal in the same translation reading frame to the nucleic acid fragment which encodes the

single chain monoclonal antibody in construct 4, and deleting the trans-activation peptide, to yield a modified construct (6), such that a resulting single chain monoclonal antibody fusion reagent may be expressed in bio-active form and bind the corresponding] wherein said trans-activation peptide in said construct 5 is deleted to yield a construct (6).

4. (Amended) The method [of] according to claim 2, wherein [the transcriptional] said transcription associated biomolecule is selected from the group consisting [essentially] of a transcription factor, ligand, hormone, nuclear hormone receptor, DNA binding domain of a nuclear hormone receptor, tumor associated protein, protein kinase, protein phosphatase, GTP binding protein, adaptor protein, secondary messenger of an intracellular signaling molecule, and a protein derived from an etiological agent.

5. (Amended) The method [of] according to claim 4, wherein [the transcriptional] said transcription associated biomolecule is selected from the group consisting of Ras, Grb2, phospholipase Cγ[-PLCγ], phosphatidylinositol 3-kinase[-PI3K], Syp, mitogen activated protein kinase[-MAPK], Jun kinase [JNK], androgen receptor [(AR)], thyroid hormone receptor [(TR)], glucocorticoid receptor[(GR)], ATF-I, ATF-2, ATF-3, ATF-4, ATF-6, CREB and CREM.

6. (Amended) The method [of] according to claim 3, wherein [the transcriptional] said transcription associated biomolecule is selected from the group consisting [essentially] of a transcription factor, ligand, hormone, nuclear hormone receptor, DNA binding domain of a nuclear hormone receptor, tumor associated protein, protein kinase, protein phosphatase, GTP binding protein, adaptor protein, secondary messenger of an intracellular signaling molecule, and a protein derived from an etiological agent.

7. (Amended) The method [of] according to claim 6, wherein [the transcriptional] said transcription associated biomolecule is selected from the group consisting of Ras, Grb2, phospholipase Cγ[-PLCγ], phosphatidylinositol 3-kinase[-PI3K], Syp, mitogen activated protein kinase[-MAPK], Jun kinase [JNK], androgen receptor [(AR)], thyroid hormone receptor [(TR)], glucocorticoid receptor[(GR)], ATF-I, ATF-2, ATF-3, ATF-4, ATF-6, CREB and CREM.

9. (Amended) A single chain monoclonal antibody fusion reagent comprising a single chain antibody fused to a trans-activation peptide, wherein said fusion reagent [capable of binding a transcriptional] binds a transcription associated biomolecule [*in vivo* isolated] within a host cell and is coded by a nucleic acid molecule produced by a method comprising:

(a) cloning a first nucleic acid fragment [which encodes a peptide DBD] that codes for a DNA-binding domain peptide of a transcription [factor] activator into [an] a first expression vector to yield a construct (1), wherein said DNA-binding domain peptide [such that the DBD may be expressed in a bio-active form and bind corresponding] binds to a DNA regulatory sequence binding site [in a heterologous host cell,];

[fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule into construct 1, in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2),]

(b) fusing a second nucleic acid fragment into said construct (1), in the same translation reading frame as the first nucleic acid fragment, to yield said first expression vector containing a construct (2) that encodes a chimeric DNA-binding domain/transcription associated biomolecule, wherein said second nucleic acid fragment codes for an antigenic portion of said transcription associated biomolecule that is sufficient to generate antibody capable of binding to said transcription associated biomolecule;

(c) providing said host cell containing a detectable gene that is under the transcriptional control of the DNA regulatory sequence binding site for said DNA-binding domain peptide;

(d) cloning a third DNA fragment that codes for a single chain antibody [an sFv library] into a second expression vector [a DNA construct] to yield a construct (3), [such that a] wherein said single chain [monoclonal antibody may be] antibody is expressed in a bio-active form [and bind a corresponding antigen in a heterologous host cell] that may bind to said antigenic portion;

(e) fusing a fourth nucleic acid fragment [which encodes] that codes for a trans-activation peptide into said construct (3), in the same translation reading frame [of] as the third nucleic acid fragment [which encodes the single chain monoclonal antibody,] to yield [a] said expression vector containing a construct (4), [such that a resulting] encoding a chimeric [sFv] single chain antibody/trans-activation peptide [may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell,] that may bind to said antigenic portion;

[providing a heterologous host cell harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2,]

(f) introducing [constructs 2 and 4] said first and second expression vectors into [the heterologous] said host cell [harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2], such that both [constructs may be] vectors are expressed [, and];

[identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule in vivo by selecting for expression of the detectable gene]

(g) monitoring the expression of said detectable gene, whereby detecting said expression indicates that said fusion reagent does bind said antigenic portion within said host cell upon detection of said expression[,];

[identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule *in vivo* by selecting for expression of the detectable gene,] and

(h) isolating [the single chain monoclonal antibody fusion reagent capable of binding the transcriptional associated biomolecule in vivo] said fusion reagent.

10. (Amended) The single chain monoclonal antibody fusion reagent [of] according to claim 9, [further comprising] said method further comprises fusing into said construct (4) at least one nucleic acid fragment [which encodes] that codes for an intracellular targeting signal peptide, in the same translation reading frame [to the] as said third nucleic acid fragment [which encodes the single chain [monoclonal] antibody

in construct 4, to yield a modified construct (5) such that a resulting single chain monoclonal antibody fusion reagent may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell], to yield a construct (5), wherein said intracellular targeting signal peptide directs the expression of said single chain antibody to a cellular compartment, whereby said reagent further comprises said intracellular targeting signal peptide fused therewith.

11. (Amended) The single chain monoclonal antibody fusion reagent [of] according to claim 9, [further comprising fusing at least one nucleic acid fragment, which encodes an intracellular targeting signal in the same translation reading frame to the nucleic acid fragment which encodes the single chain [monoclonal] antibody in construct 4, and deleting the trans-activation peptide, to yield a modified construct (6), such that a resulting single chain monoclonal antibody fusion reagent may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell], wherein said trans-activation peptide in said construct 5 is deleted to yield a construct (6).

12. (Amended) [A] The single chain monoclonal antibody fusion reagent according to claim 9, [which] wherein said reagent is capable of regulating transcription [*in vivo*] in said host cell.

13. (Amended) [A] The single chain monoclonal antibody fusion reagent according to claim 10, [which] wherein said reagent is capable of regulating transcription [*in vivo*] in said host cell.

14. (Amended) [A] The single chain monoclonal antibody fusion reagent according to claim 11, [which] wherein said reagent is capable of regulating transcription [*in vivo*] in said host cell.

15. (Amended) A therapeutic method for regulating the transcription of a gene [*in vivo* comprising] associated with a condition or symptom comprises administering an effective amount of a single chain monoclonal antibody fusion reagent [capable of binding a transcriptional] that targets a transcription associated biomolecule [*in vivo* identified by a method] within a host cell, wherein said fusion reagent is prepared by the steps, comprising:

(a) providing [an expression construct (1)which encodes a peptide DBD of a transcription factor and comprises a cloning site for fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2),] a first expression vector comprised of (i) a first nucleic acid fragment that codes for a DNA binding domain peptide of a transcription activator that binds a DNA regulatory sequence binding site, and (ii) a second nucleic acid fragment that codes for an antigenic portion of said transcription associated biomolecule that is sufficient to generate antibody capable of binding to said transcription associated biomolecule, wherein said first and said second fragments are in the same translation reading frame, whereby said first expression vector encodes a chimeric DNA-binding domain/transcriptional associated biomolecule;

(b) providing said host cell containing a detectable gene that is under the transcriptional control of the DNA regulatory sequence binding site for said DNA-binding domain peptide;

(c) providing a second expression vector [DNA construct (3) which encodes a trans-activation peptide and comprises a cloning site for fusing an sFv library in the same translation reading frame of the trans-activation peptide to yield a construct (4), such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind a transcriptional associated biomolecule in the heterologous host cell,] that comprises (i) a third nucleic acid fragment that codes for a single chain antibody that is expressed in a bio-active form that may bind to an antigen present in said host cell and (ii) a fourth nucleic acid fragment that codes for a trans-activation peptide, wherein said third and fourth fragments are in the same translation reading frame, whereby said second expression vector encodes a chimeric single chain antibody/trans-activation peptide that may bind to said antigenic portion;

[providing a the heterologous host cell, harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2,]

(d) [for] introducing [constructs 2 and 4 into the heterologous] said first and second expression vectors into said host cell, such that both [constructs may be] vectors are expressed, and

[identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule *in vivo* by selecting for expression of the detectable gene]

(e) monitoring the expression of said detectable gene, whereby detecting said expression indicates that said fusion reagent does bind said antigenic portion within said host cells upon detection of said expression.

16. (Amended) [A] The therapeutic method [for regulating the transcription of a gene *in vivo*] according to claim 15, wherein [the single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule *in vivo* comprises] said fusion reagent is fused to at least one nucleic acid fragment encoding an intracellular targeting signal peptide.

17. (Amended) A method of screening a plurality of compounds for specific binding affinity with a single chain monoclonal antibody fusion reagent according to claim 9 [capable of binding a transcriptional associated biomolecule *in vivo*] within a host cell [identified by a method], comprising:

[providing an express ion construct (1) which encodes a peptide DBD of a transcription factor and comprises a cloning site for fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2),

providing a DNA construct (3) which encodes a trans-activation peptide and comprises a cloning site for fusing an sFv library in the same translation reading frame of the trans-activation peptide, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind a transcriptional associated biomolecule in a heterologous host cell,

providing a heterologous host cell, harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding Site corresponding to the DBD encoded by construct 2, for introducing constructs 2 and 4 into the heterologous host cell, such that both constructs may be expressed, and

identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule *in vivo* by selecting for expression of the detectable gene, and

screening a plurality of compounds comprising the steps of:]

(a) providing a plurality of compounds[,];

(b) [combining] contacting said [single chain monoclonal antibody] fusion reagent[,] with each of [a] said plurality of compounds for a time sufficient to allow binding under suitable conditions; and

(c) detecting [binding of said single chain monoclonal antibody fusion reagent to each of the plurality of compounds, thereby identifying the] at least some compounds of said plurality that [which] specifically binds [said] to said single chain monoclonal antibody fusion reagent.

18. (Amended) A method for diagnosing a physiological disorder manifested by abnormal levels of a transcription associated biomolecule, said method comprising:

(a) contacting a biological sample containing said transcription associated biomolecule with a [labelled] labeled single chain monoclonal antibody fusion reagent according to claim 9 [or a portion thereof claim 9], whereby said [antibody] reagent binds to said transcription associated biomolecule to form a complex[,];

(b) separating an unbound [labelled antibody] labeled reagent from a bound labeled reagent in said complex[,]; and

(c) measuring the amount of said unbound and bound [labelled] labeled [antibody reagent] reagents in said complex under identical conditions.

[comparing the quantity of labelled antibody reagent in said biological sample tothe quantity of labelled antibody reagent which binds to normal biological samples under identical conditions.]

19. (Amended) A pVP16Zeo library expression vector, accorded as ATCC Accession [(ATCC deposit #___)] No. 98483, for the construction and screening of a single chain monoclonal antibody fusion reagent [libraries], comprising a zeocin

[selection] selective marker gene to facilitate the isolation and production of said single chain monoclonal antibody fusion reagent[s] in yeast and *E. coli.*

20. (Amended) A kit for screening a DNA construct library for a single chain monoclonal antibody fusion reagent [capable of binding a transcriptional associated biomolecule *in vivo;* in a heterologous host cell, comprising in a container] within a host cell, comprising:

(a) [an expression construct (1) which encodes a peptide DBD of a transcription factor and comprises a cloning site for fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2), and] a first expression vector comprised of (i) a first nucleic acid fragment that codes for a DNA binding domain peptide of a transcription activator that binds a DNA regulatory sequence binding site, and (ii) a second nucleic acid fragment that codes for an antigenic portion of a transcription associated biomolecule, wherein said first and said second fragments are in the same translation reading frame, whereby said first expression vector encodes a chimeric DNA-binding domain/transcriptional associated biomolecule;

(b) a [DNA construct (3) which encodes a trans-activation peptide and comprises a cloning site for fusing an sFv library in the same translation reading frame of the trans-activation peptide, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind a transcriptional associated biomolecule in the heterologous host cell,] second expression vector that comprises (i) a third nucleic acid fragment from said DNA construct library that codes for a single chain antibody that is expressed in a bio-active form that may bind to said antigenic portion, and (ii) a fourth nucleic acid fragment that codes for a trans-activation peptide, wherein said third and fourth fragments are in the same translation reading frame, whereby said second expression vector encodes a chimeric single chain antibody/trans-activation peptide that may bind to said antigenic portion;

[a heterologous host cell, harboring a detectable gene under the transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct (2); and]

(c) said host cell containing a detectable gene that is under the transcriptional control of the DNA regulatory sequence binding site for the DNA-binding domain peptide, for introducing said first and second expression vectors such that both vectors are expressed;

(d) [a] means for [identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule *in vivo* by selecting for expression of the detectable gene] monitoring the expression of said detectable gene, whereby detecting said expression indicates that said fusion reagent does bind said antigen present in said host cell.

21. (Amended) A kit [for screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcription associated biomolecule *in vivo*] according to claim 20, [wherein DNA construct 3 is] further comprises a pVPI6Zeo [(ATCC deposit # __)] vector, wherein said vector expresses said single chain antibody and is accorded as ATCC Accession No. 98483.

22. (Amended) A kit [for screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule [*in vivo*] in a heterologous host cell] according to claim 21, further comprises primers, wherein said primers are [provided for human sFv library construction] selected from the group consisting of SEQ ID NOS:3 – 86.